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Obtaining of hybrids within the family *Cucurbitaceae* by in vitro culture of immature embryos. III. Characteristics of hybrids of *Cucurbita maxima* × *C. ficifolia* and *C. maxima* × *C. foetidissima*

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A b s t r a c t. Two wild species, *Cucurbita foetidissima* and *C. ficifolia*, were reciprocally crossed with the cultivar *C. maxima*. Fruits were obtained only when *C. maxima* was used as a maternal component. In order to obtain hybrid plants it was necessary to use in vitro culture of immature embryos. Colchicine treatment was applied for doubling the chromosome number. Hybrids were male-sterile and partialy female-fertile. They were characterized on the basis of morfological traits of plants and fruits and on the basis of an analysis of isoenzymes – esterases and peroxidases. Morfological traits of plants of paternal components were mostly dominant. The degree of fruit setting after crossing and the degree of pollen grain stainability were influenced by maternal components. Heterosis effect was observed in the intensity of photosynthesis.

Key words: *Cucurbita ficifolia*, *Cucurbita foetidissima*, *Cucurbita maxima*, embryo culture, interspecific hybrids.

Interspecific hybridization is one of the ways permitting to introduce new characters into plants, such as disease resistance, resistance to unfavourable environmental conditions, yield reduction and others. However, this possibility is limited by the existing crossability barriers, which makes necessary to search for different methods to overcome them. Crossability barriers in the genus *Cucurbita* usually have a postzygotic nature (RAKOCZY-TROJANOWSKA, MALEPSZY 1986). It is possible to overcome them by using in vitro culture of

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immature embryos (PITRAT, de VAULX 1980, NIJST, OOST 1980, RAKOCZY-TROJANOWSKA, MALEPSZY 1986, 1989). This technique allowed to obtain a lot of interspecific hybrids, such as: *Cucurbita maxima* × (*C. pepo* × *C. moschata*) with fruits of a good taste (BIELIK, PODMOGAEVA 1957, 1960), *C. maxima* × *C. moschata* with a high content of carotenoides (ANDRIYASH 1981), *C. pepo* × *C. ecuadorensis* resistant to disease, *C. martinenzii* × *C. pepo* with a complete resistance to CMV and partial resistance to powdery mildew (PITRAT, de VAULX 1980). Other species of interest for breeding programs were *C. ficifolia* and *C. foetidissima*. The first of them has a valuable high resistance to soil-borne diseases (WHITAKER, ROBINSON 1986), and the second one is resistant to many viruses, drought and distinguishes by a high level of carbohydrates in shoots (WHITAKER, ROBINSON 1986). *C. maxima* used in the crosses has a bushy type of growth, and high contents of dry matter and beta-caroten in fruits. The objective of the presented work was to introduce these traits into the cultivars of *C. maxima* species.

Material and methods

Interspecific hybridizations between *Cucurbita maxima*, *C. ficifolia* and *C foetidissima* were carried out in 1990 and 1991. Two inbred lines of *C. maxima* No. 62 and 274, both obtained at our Department – were used as plant material. *C. ficifolia* from the same source and *C. foetidissima* were obtained from the National Institute of Agrobiological Resource (No. 78105002-OLD-670005).

Crosses between *C. maxima* 62 and *C. ficifolia* were made in the greenhouse, whereas those between *C. maxima* 274 and *C. foetidissima* and *C. ficifolia*, *C. maxima* 62 and *C. foetidissima* as well as backcrosses were performed in the field. The methods of embryo isolation and sterilization were the same as previously described (RAKOCZY-TROJANOWSKA, MALEPSZY 1986), except the period after pollination, when fruits were harvested. Embryos from *C. maxima* 62 × *C. ficifolia* were isolated 40-45 days after pollination. The medium used for embryo culture contained macro- an microelements as described by MURASHIGE and SKOOG (1962), 250mg l^{-1} of edamine, 7% Difco agar, 30g l^{-1} of sacharose, pH 5.7-5.8. Other conditions of in vitro culture were the same as in RAKOCZY-TROJANOWSKA and MALEPSZY (1986). All hybrids were vegetatively propagated, and finally each of them was represented by 4-6 plants. Half of them were then treated with colchicine as described by WHITAKER and ROBINSON (1986).

The following morfological traits of hybrids were characterized: the type of plant growth, shape and size of leaves, fruit shape and colour. The resistance to powdery mildew was determined visually, based on increasing level of infection. Early stages of micro- and macrosporogenesis were investigated by the previously described methods (RAKOCZY-TROJANOWSKA, MALEPSZY 1986). Five male flowers were taken from each combination and three samples were prepared from each of them. Examination of PER isozymes followed SHAW and PRASARD (1970), and ETS – RUDIN and RASMUSON (1973). The intensity of photosynthesis was determined by mean Li-cor 6200, using mature leaves for measurement.

Results

Efficiency of pollination and embryo culture

Crosses were carried out in both directions, but fruits were set only in the case when *C. maxima* was used as a maternal component, and the line 274 proved to be better than 62 (Table 1). There were no significant differences between the crosses in fruit setting. Almost three times more embryos developed into plants in crosses where *C. maxima* 274 was used as a maternal component than in crosses with the second line. The number of embryos developing into plants ranged from 47.6% in crosses with *C. ficifolia* to 53.4% in crosses with *C. foetidissima* (Table 2). Some abnormally developing embryos

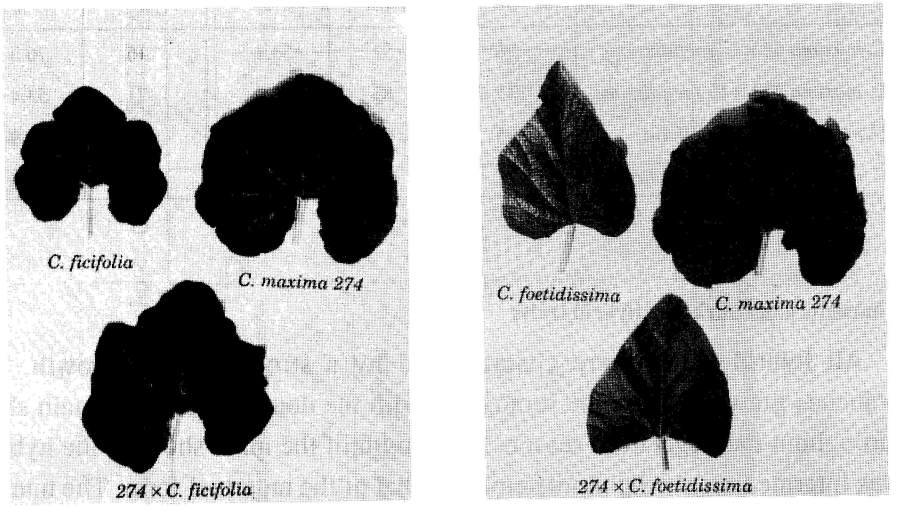

Fig. 1. A comparision of leaves shape of the hybrids: *C. maxima* × *C. ficifolia* and *C. maxima* × *C. foetidissima*

Table 1. The efficiency of cross pollination *C. maxima* × *C. foetidissima* and *C. maxima* × *C. ficifolia*

Cross combination	No. of crosses	Fruit set (%)	Embryos No.		
			totaly	per cross	per fruit
C. maxima 62 × *C. foetidissima*	28	32.1	133	4.8	14.8
C. maxima 274 × *C. foetidissima*	19	26.3	98	5.2	19.6
C. foetidissima × *C. maxima* 62	10	0	–	–	–
C. foetidissima × *C. maxima* 274	12	0	–	–	–
C. maxima 62 × *C. ficifolia*	33	18.2	95	2.9	15.8
C. maxima 274 × *C. ficifolia*	21	23.8	21	4.7	19.6
C. ficifolia × *C. maxima* 62	16	0	–	–	–
C. ficifolia × *C. maxima* 274	11	0	–	–	–

were encountered in the cross *C. maxima* 62 × *C. ficifolia*. They lacked roots, top meristems or had modified leaves, and died after few weeks of in vitro culture.

Table. 2. Hybrids of *C. maxima* × *C. foetidissima* and *C. maxima* × *C. ficifolia* embryo rescue culture in vitro

Hybrid	Cultured embryos No.	Embryos starting development		Embryos developing into plants	
		No.	%	No.	%
C. maxima 62 × *C. foetidissima*	98	52	53.1	16	16.3
C. maxima 274 × *C. foetidissima*	133	83	62.4	71	53.4
C. maxima 62 × *C. ficifolia*	95	62	60.4	18	18.9
C. maxima 274 × *C. ficifolia*	21	10	47.6	10	47.6

Characteristics of chosen hybrid traits

All hybrid plants were characterized by a strong type of growth. The branches grew out from the root collar with the dominance of the main shoot throughout the vegetation season. The length of the main shoot of the hybrids was intermediate, being more similar to that of the maternal form. The number of branches was similar and smaller in all the cases than in the parental plants (Table 3).

Table. 3. Characteristics of chosen *C. maxima* × *C. ficifolia* and *C. maxima* × *C. foetidissima* hybrid traits

Hybrids and parental components	Number of plants	Type of growth						Leaf shape	Fruit		Resistance to powdery mildew*
		visually	shoot number		length of main shoot (cm)		number of plants with the main shoot		shape	colour	
			mean	range	mean	range					
C. maxima 274 × *C. ficifolia*	56	strong	1.67	1-4	195.0	50-400	40	p/*fici*	*fici*	*fici*	2
C. maxima 274 × *C. foetidissima*	22	"	1.55	1-3	190.0	80-250	14	p/*foe*	*foe*	*foe*	1
C. maxima 62 × *C. foetidissima*	123	"	1.84	1-4	209.0	100-350	115	p/*foe*	*foe*	*foe*	4
C. maxima 62 × *C. ficifolia*	18	"	2.44	1-4	176.0	50-280	15	p/*fici*	*fici*	*fici*	3
C. maxima 62	20	"	2.6	–	94.0	–	–	–	–	–	5
C. maxima 274	20	"	2.8	–	110.0	–	–	–	–	–	5
C. foetidissima	10	"	4.2	–	606.0	–	–	–	–	–	1
C. ficifolia	10	"	6.0	–	778.0	–	–	–	–	–	2

fici – similar to *C. ficifolia*, *foe* – similar to *C. foetidissima*, p – intermediate

* 1–5 scale of increasing powdery mildew infection

Table 4. Characteristics *C. maxima 62* × *C. foetidissima* fruits from colchicine-treated and non treated plants

Colchicine	Fruit length (cm)		Fruit width (cm)		Carpophore length (cm)	
	mean	range	mean	range	mean	range
Non treated	36.5	20-40.5	36.1	28-42	3.8	3.0-5.4
Treated	33.7	28.0-40.0	32.9	24.8-40.8	4.2	2.2-5.0

A comparision of fruit shape and colour between colchicine-treated and untreated plants proved, that in the first case the fruit length and width were smaller. Only the carpophore of the fruits was longer (Table 4).

The intensity of photosynthesis in all hybrids was higher than in the parents. The ratio between chlorophyl a and b and their contents were comparable in all the forms (Table 5).

Table 5. Characteristics of photosynthesis in *C. maxima* × *C. ficifolia* and *C. maxima* × *C. foetidissima* hybrids

Hybrids and parental components	Chlorophyl concentration in plant (mg $1000cm^{-3}$)		Chlorophyl a:b ratio	Photosynthesis efficiency ($\mu mCO_2 m^{-2} s^{-1}$)
	a	b		
C. maxima 274 × *C. ficifolia*	17.76	9.3	1.9	16.58
C. maxima 274 × *C. foetidissima*	16.9	9.31	1.82	20.62
C. maxima 62 × *C. foetidissima*	18.51	11.57	1.6	21.62
C. maxima 62 × *C. ficifolia*	nd	nd	nd	nd
C. maxima 62	16.23	8.22	1.98	11.53
C. maxima 274	14.8	6.87	2.15	15.08
C. foetidissima	17.44	9.42	1.85	13.17
C. ficifolia	19.57	11.48	1.71	11.64

nd – not determined

An examination of microsporogenesis showed that this process was strongly disturbed. Meiotic divisions of pollen mother cells (PMC) were irregular, which resulted in different size of pollen grains (PG) (Fig. 2-5). The number of regular PG was similar in hybrids of the both species and equalled 29.1-34.9%, with the exception of *C. maxima* 62 × *C. ficifolia*, where the percentage of normal PG was higher – 88.3% (Table 6). An additional estimation of male fertility

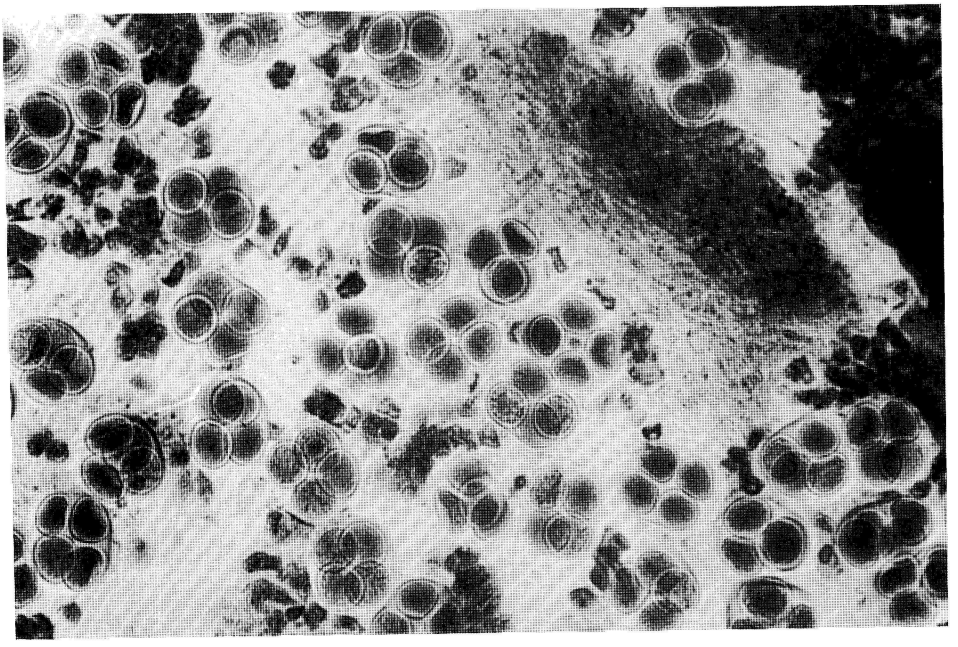

Fig. 2. Regular division of PMC in *C. maxima* (maternal component)

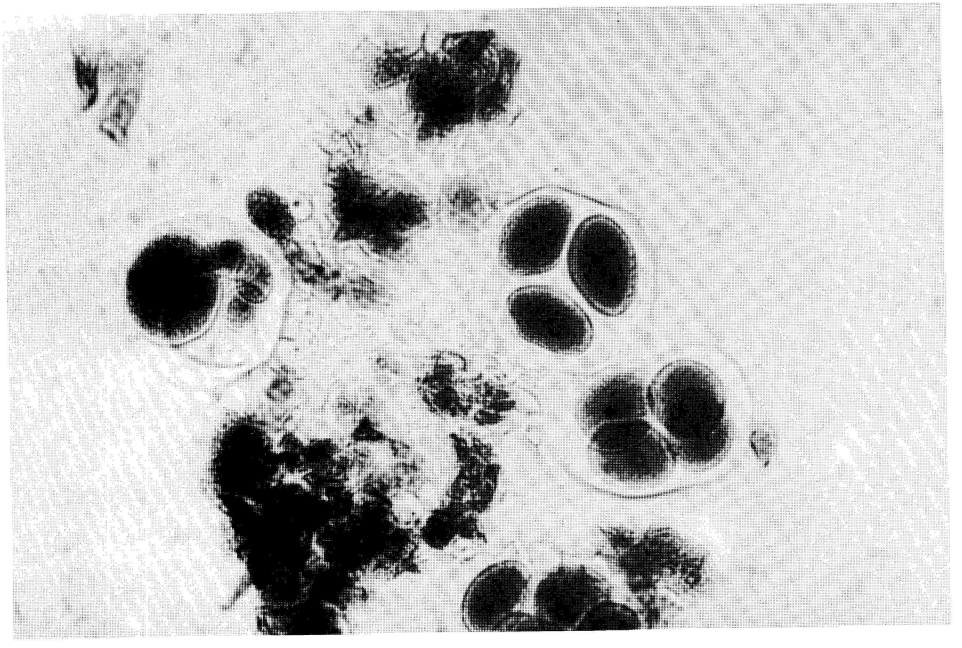

Fig. 3. Irregular division of PMC in the hybrids *C. maxima* × *C. foetidissima*

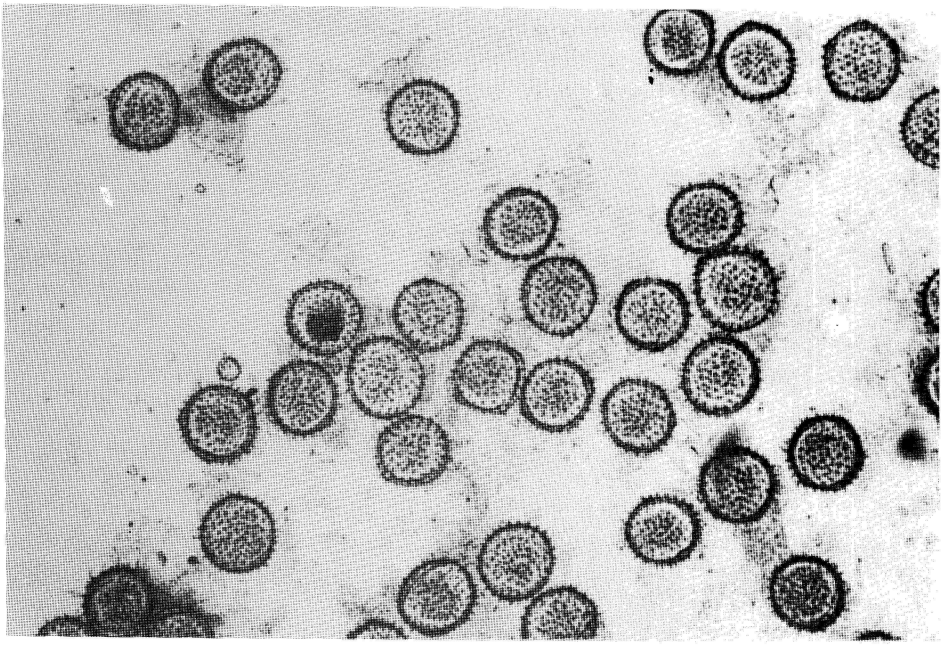

Fig. 4. Regular PG of *C. maxima*

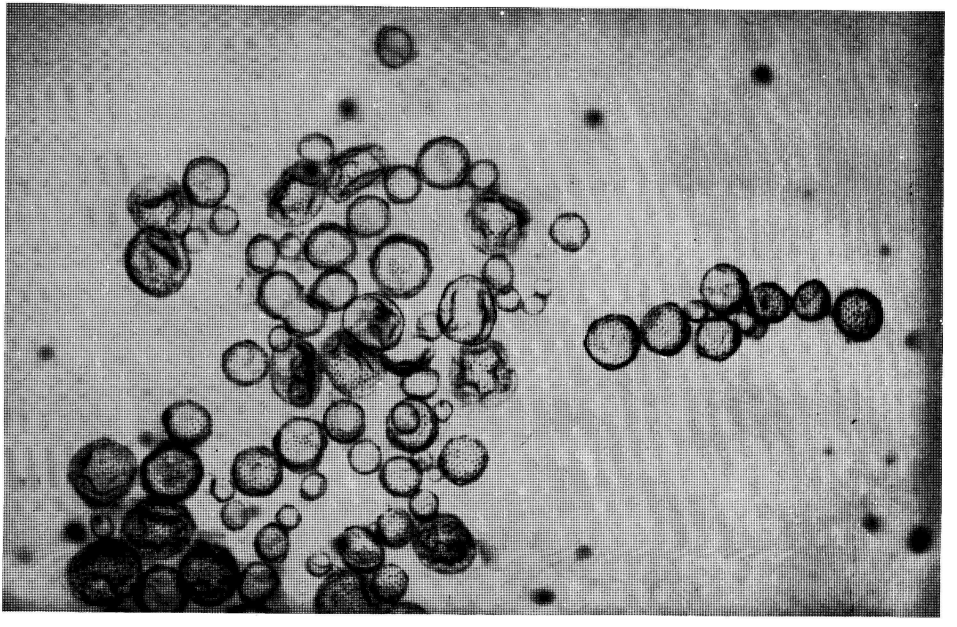

Fig. 5. Irregular PG of the hybrids *C. maxima* × *C. foetidissima*

Table. 6. Characteristics of pollen grains of *C. maxima* × *C. ficifolia* and *C. maxima* × *C. foetidissima* hybrids

Hybrids and parental components	Pollen grains (%)			
	small	huge	small and stainable	small and nonstainable
C. maxima 62 × *C. foetidissima*	30.8	0	2.8	66.4
C. maxima 274 × *C. foetidissima*	34.9	1.9	21.1	41.9
C. maxima 62 × *C. ficifolia*	88.3	4.8	4.3	2.6
C. maxima 274 × *C. ficifolia*	29.1	2.7	3.7	64.5
C. maxima 62	95.8	0	3.9	2.3
C. maxima 274	97.8	0	0.4	1.8
C. foetidissima	72.9	0	0.5	26.8
C. ficifolia	94.6	0.3	0.7	4.4

was based on results of backcrosses where hybrids were used as male components.

All hybrids which belonged to the same cross showed identical isozyme patterns. When analysing enzymes of peroxidases (PER), 17 zones were distinguished for *C. maxima* × *C. ficifolia* and 11 zones for *C. maxima* × *C. foetidissima*. An analysis of esterases (EST) for all hybrids made it possible to distinguish 10 zones. Table 7 summarizes observations concerning the occurrence of the same patterns as in the both parents, maternal or paternal forms and new bands as compared to parents or no bands.

Discusion

Obtaining of hybrids

This work has confirmed that in vitro culture makes it possible to obtain interspecific hybrids within *Cucurbitaceae* family. An important factor responsible for good response was the age of isolated embryos. They were up to twenty days older in comparision with *C. maxima* and *C. pepo* hybrids. The efficiency was determined by the number and percentage of set fruits, by the number of embryos per cross and per fruit. The data for *C. maxima* × *C. ficifolia* cross were comparable to those of WEILING (1955). In this case the number of obtained plants and the number of embryos per fruit were three times higher.

Table. 7. Isozymes patterns in hybrids *C. maxima* × *C. foetidissima* and *C. maxima* × *C. ficifolia*

Hybrid	Enzyme and zone	The same pattern as in			New bands	No bands
		both parents	maternal compo-nent	paternal compo-nent		
C. maxima × *C. foetidissima*	EST 1	+				
	2					+
	3	+				
	4		+			
	5					+
	6		+			
	7					
	8					+
	9					
	10	+	+			
	PER 1	+				
	1			+		
	2					
	3		+			
	4					+
	5	+				
	6			+		
	7					+
	8					+
	9					+
	10			+		
	11					+
	12			+		
	13					+
	14			+		
	15		+			
	16	+				
	17					+
C. maxima × *C. ficifolia*	EST 1					+
	2		+			
	3					+
	4	+				
	5		+			
	6			+		
	7					+
	8	+				
	9					+
	10					+
	PER 1	+				
	2			+		
	3		+			
	4					+
	5	+				
	6				+	+
	7					
	8	+				
	9			+		
	10	+				
	11	+		+		

+ presence of band, PER – peroxydases, EST – esterases

Characteristics of hybrids

The number of shoots, their length and the shape of leaves were intermediate as compared to those of the parental forms. Only leaves of *C. maxima* 62 × *C. ficifolia* from greenhouse reminded the maternal plants. PEARSON et al. (1951) examined hybrids *C. moschata* × *C. maxima* and described their shoots and leaves as intermediate but more similar to the maternal component.

During the vegetation, disease resistance, mainly to powdery mildew, was estimated. The hybrids were more resistant then the maternal forms, but more susceptible when compared to the paternal forms. PITRAT and de VAULX (1980) found that most of wild species, including *C. foetidissima* are resistant to this pathogen.

The hybrids obtained in this experiment were male-sterile and our examinations showed, that the male sterility was functional. Male flowers or anthers did not open.

The fraction of well-stained PG of all hybrids exceeded 35.5% amounting to 97.4%. The stainability of the parental PG was high, more than 95%, except *C. foetidissima*, where it was 73.4%.

PEARSON et al. (1951) examined hybrids from *C. maxima* × *C. moschata* and obtained similar results. The stainability of PG of the parental forms exceeded 95%.

Hybrids obtained by GROFF and BEMIS (1967) (*C. moschata* × *C. lundelliana*) × *C. foetidissima* were sterile, which is in desagreement with our studies. These authors found that meiosis was strongly disturbed. It was irregular and instead of bivalents there appeared numerous univalents (27.6 ± 5.3%). As a consequence, different numbers of PG were obtained from single PMC. In all hybrids the anthers did not open. Similar results were obtained by RAKOCZY-TROJANOWSKA and MALEPSZY (1986) with *C. maxima* × *C. pepo* hybrids.

Colchicine treatment has not solved this problem, which indicates that this could be an effect of a changing action of some genes responsible for PG functioning. The hybrids of *C. maxima* × *C. foetidissima* (and *C. ficifolia*) were partialy female-fertile. An examination of embryogenesis after backcrosses, when hybrids were used as maternal components, showed the presence of embryo at the heart stage in one case, and the presence of nuclear endosperm and an indication of pollen tube penetration. The indication of pollen tube penetration proved that there could be also postzygotic barriers. It is possible that in these few cases the fertilization was normal, but the embryos and endosperm died in early developmental stages. KWACK and FUJIEDA (1985) observed that hybrids between *C. maxima*, *C. moschata* and *C. pepo* set seeds

almost normally, but the seeds had no embryos. This indicates that there are some barriers in *Cucurbita* genus.

The efficiency of photosynthesis, was higher in hybrids than in the parental forms. The highest efficiency was observed, when *C. foetidissima* was used as a paternal form. In this case the efficiency almost doubled the value of the parental components.

One of the best methods of hybrid characterization is a biochemical analysis of isozymes used by WEEDEN and GRAHAM (1986), KIRKPATRICK et al. (1985), RAKOCZY-TROJANOWSKA and MALEPSZY (1986), RAKOCZY-TROJANOWSKA et al. (1992). The EST and PER were the most useful isozymes for discrimination of our hybrids. Bands, characteristic only of hybrids could be easily seen. Instead of them, bands similar to those of the both parental forms could be distinguished in F_1 plants. No differences in bands migration were found between the plants growing in the field and in the greenhouse. IGNART and WEEDEN (1984) obtained different results. In their experiments, environmental conditions influenced the band migration. We tried to characterize other enzymes, such as malate dehydrogenase or isocitric dehydrogenase. Although they were useful in similar experiments performed by IGNART and WEEDEN (1984), KIRKPATRICK et al. (1985), WEEDEN and GRAHAM (1986), our studies have not confirmed that. The obtained bands were very weak and on that account it was difficult to read zygograms.

The obtaining of hybrids within the *Cucurbitaceae* family is very complicated and depends on many factors, but as shown above it is possible using in vitro culture of immature embryos.

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Otrzymywanie mieszańców w obrębie rodziny *Cucurbitaceae* poprzez kulturę in vitro niedojrzałych zarodków. III. Charakterystyka mieszańców między gatunkami *Cucurbita maxima* × *C. ficifolia* i *C. maxima* × *C. foetidissima*

Streszczenie

Dwa dzikie gatunki, *C. foetidissima* i *C. ficifolia* były wzajemnie krzyżowane z formą uprawną *C. maxima*. Owoce otrzymano tylko wtedy, gdy jako komponenta matecznego użyto *C. maxima*. W celu otrzymania roślin mieszańcowych konieczne było zastosowanie kultury in vitro niedojrzałych zarodków. Liczbę chromosomów u mieszańców podwajano za pomocą

kolchicyny. Rośliny mieszańcowe były męskosterylne i częściowo żeńskopłodne. Scharakteryzowano je na podstawie cech morfologicznych oraz analizy izoenzymów: esteraz i peroksydaz. Analizowano także intensywność fotosyntezy u mieszańców i form rodzicielskich. Stwierdzono, że cechy morfologiczne komponentów ojcowskich były w większości dominujące. Komponenty mateczne miały wpływ na zawiązywanie owoców i stopień barwliwości ziaren pyłku. U wszystkich mieszańców stwierdzono zjawisko heterozji w odniesieniu do procesu fotosyntezy.